Biol Blood Marrow Transplant 21 (2015) 1215–1222

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Biology: Biomarkers

Peripheral Blood CD38 Bright CD8$^+$ Effector Memory T Cells Predict Acute Graft-versus-Host Disease

Pooja Khandelwal [1,*], Adam Lane [1,2], Vijaya Chaturvedi [1], Erika Owsley [1], Stella M. Davies [1], Daniel Marmer [3], Alexandra H. Filipovich [1], Michael B. Jordan [1,4], Rebecca A. Marsh [1]

[1] Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[2] Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[3] Cancer and Blood Diseases Institute Laboratory, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[4] Division of Immunobiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

Article history:
Received 27 February 2015
Accepted 7 April 2015

Key Words:
CD38 bright CD8$^+$ effector memory T cells (TEMs)
Effector memory T cells
Graft-versus-host disease (GVHD) predictive biomarker

A B S T R A C T

Acute graft-versus-host disease (aGVHD) is mediated by allogeneic T cell responses. We hypothesized that increases of peripheral blood–activated CD8$^+$ effector memory T (TEM) cells would be observed after hematopoietic stem cell transplantation (HSCT) before onset of aGVHD symptoms. Blood was collected twice weekly after HSCT for 7 weeks in 49 consecutive pediatric and adult HSCT recipients. Samples were incubated with fluorochrome-conjugated antibodies against CD45, CD3, CD8, CD38, CD45RA, and CCR7 and analyzed using flow cytometry. TEM cells were defined as CD3$^+$ CD8$^+$CCR7$^-$ CD45RA$^-$ lymphocytes. CD38 expression was used as a marker of T cell activation. Patients were followed for 100 days for development of aGVHD. Twenty-three patients developed grade 1 to 4 aGVHD at a median of 37 days (range, 15 to 79 days) after HCST. Absolute CD38 bright CD8$^+$ TEM of $>$ 35 cells/μL predicted aGVHD at a median of 8 days (range, 1 to 34) before aGVHD onset with a sensitivity of 82.6% and specificity of 91.6%. The cumulative incidence of aGVHD was 90% in patients with absolute CD38 bright CD8$^+$ TEM $>$35 cells/μL and 15% in patients without ($P <$ .0001). Quantification of CD38 bright CD8$^+$ TEM cells may predict aGVHD in children and young adult HSCT recipients.

INTRODUCTION

Acute graft-versus-host disease (aGVHD) is a significant complication of allogeneic hematopoietic stem cell transplantation (HSCT) and is the leading cause of nonrelapse mortality [1]. Approximately 35% to 50% of HSCT recipients develop aGVHD; however, there are no validated predictive blood biomarkers for aGVHD in clinical use [1,2]. The ability to predict aGVHD before onset may allow for development of pre-emptive therapeutic strategies that could potentially reduce post-transplantation morbidity and mortality related to aGVHD [3]. Various biomarkers in blood or urine have been investigated, but their applicability is limited by suboptimal sensitivity or specificity, elevated cost of processing samples, or lack of validation in large-scale clinical trials (Table 1) [4-15]. Thus, a need exists for novel, noninvasive biomarkers that can reliably predict aGVHD.

T cells are known to play a role in both acute and chronic GVHD, but the exact subset of T cells implicated in the pathogenesis of GVHD in humans remains unclear [16-19]. T cells are classically divided into naïve and memory cells, based on the presence or absence of previous antigen encounter. After antigen exposure, naïve T cells have the ability to expand and differentiate into memory T cells [20]. C-C chemokine receptor type 7 (CCR7, cluster of differentiation 197) is a chemokine receptor that plays a role in homing of T cells to secondary lymphoid organs. The presence or absence of its expression divides human memory T cells into 2 functionally distinct subsets [21]. Central memory T cells (CCR7$^+$) do not possess immediate effector function [20]. These are usually found in lymphoid tissues and upon stimulation, differentiate into CCR7$^-$ effector cells [21]. Effector memory T cells (TEM) (CCR7$^-$) are excluded from lymphoid tissues, express receptors for migration to inflamed tissues, and display immediate effector function [21]. These cells rapidly produce effector cytokines or express perforin granules [21].

Financial disclosure: See Acknowledgments on page 1221.

* Correspondence and reprint requests: Pooja Khandelwal, MD, Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45208.
E-mail address: Pooja.khandelwal@cchmc.org (P. Khandelwal).

http://dx.doi.org/10.1016/j.bbmt.2015.04.010
1083-8791/

Table 1
Biomarkers Evaluated for Prediction of aGVHD

Author	Biomarker	Day of Measurement	No. of Patients	Sensitivity	Specificity	AUC
Miyamoto et al.	sIL2r	Day +3	30	Not mentioned	Not mentioned	
Kitko et al.	TNFr1	Day +7	82 children	31%	82%	.63
Willems et al.	TNFr1 in nonmyeloablative conditioning	Day +7/baseline ratio	106	Hazard ratio, 2.1; $P = .01$		
August et al.	sIL2r+ sCD8+ sTNFr1	Day +15	62	64%	76%	.77
Liu et al.	Plasma IL-6	Day 1-21 after transplantation	101	Not mentioned		
Xiao B et al.	Combined 4 miRNA panel	2 weeks after HSCT	196	92%	62%	.80
Paczesny et al.	IL2Ra, TNFr1, elafin	Preconditioning, day +7 and day +14 after HSCT	Training = 342 Validation = 142	75%	57%	
Kaiser T et al.	16 urine polypeptides	Twice weekly before conditioning until discharge	35	100%	82%	
Thiant et al.	IL7 (myeloablative conditioning)	Day +14	40	Hazard ratio, 5.38; $P = .022$		
Thiant et al.	IL7 (reduced-intensity conditioning)	Day +30	45	Hazard ratio, 1.221; $P = .002$		
Choi et al.	sTNFr1 in myeloablative conditioning	Before transplantation and day +7	438	38%	83%	
Vander Lugt et al.	ST2	Day +14	673	Predictive of nonrelapse mortality after transplantation		

AUC indicates area under the curve.

Research in murine models suggests that $CD4^+$ and $CD8^+$ naïve T cells cause GVHD [22-24]. In addition, $CD8^+$ central memory cells may also be able to mediate aGVHD [16]. In contrast, TEM cannot cause murine aGVHD unless previously primed [25,26]. After allogeneic HSCT in humans, it is possible that aGVHD responses evolve as naïve T cells are activated by alloantigens and then proliferate and differentiate into memory subsets with effector functions. The recent observation in humans of increased numbers of $CD4^+$ and $CD8^+$ TEM cells at day +28 after HSCT in patients who developed aGVHD suggests that TEMs play a role in aGVHD [27]. Interestingly, 2 early flow cytometric human studies that examined total $CD4^+$ and $CD8^+$ T cell populations in patients after allogeneic HSCT noted a predominant association of aGVHD with peripheral blood $CD8^+$ T cell populations or with decreasing CD4 to CD8 ratios, suggesting that $CD8^+$ T cell expansion plays an important role in human aGVHD [28,29].

Previous human studies have also attempted to discover a T cell surface activation marker that correlates with aGVHD without success [30]. Cluster of differentiation 38 (CD38) is located on human chromosome 4 (4p15) [31]. CD38 plays dual roles as receptor and ectoenzyme and regulates activities related to signaling and cell homeostasis [31]. CD38 was initially thought to be expressed by thymocytes and

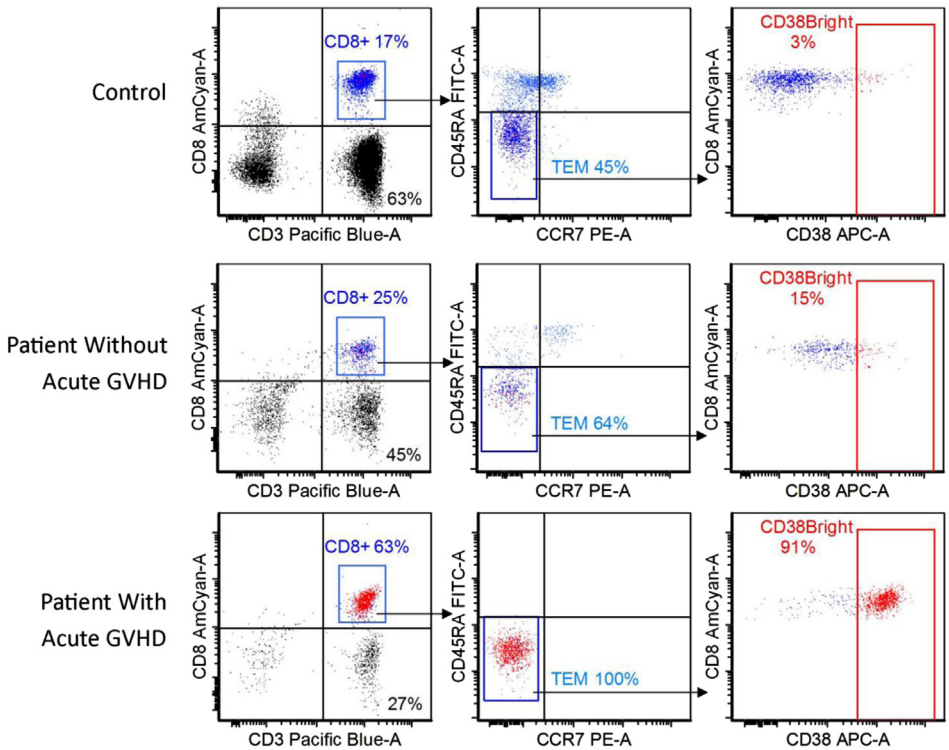

Figure 1. Representative dots plots demonstrating gating strategy for CD38 bright $CD8^+$ TEM cells in a healthy adult control (top panel), a patient after HSCT who did not develop aGVHD (middle panel), and a patient after HSCT who subsequently developed aGVHD (bottom panel). Analysis was restricted to an initial lymphocyte gate, based on CD45 intensity and side light scatter characteristics.

activated T lymphocytes and believed to play a role in activation and proliferation of human T cells [32,33], but it is now known to be present throughout the immune system with varying levels of expression [31]. CD38 plays a significant role in various disease states, namely chronic lymphocytic leukemia [34], ovarian cancer [35], and multiple myeloma [31], and its presence is associated with clinically aggressive disease and poor prognosis. In addition, high ratios of $CD8^+$ $CD38^+$ T lymphocytes predict disease progression in HIV infected adults [36,37]. Perhaps, the most relevant role of CD38 lies in its interaction with platelet endothelial cell adhesion molecule-1, which is crucial for leukocyte migration through the endothelium [38]. The role of CD38 in aGVHD has not been explored yet, but it is possible that upregulation of CD38 in activated TEMs could be an important step in trafficking of these cells to target organs.

To test our hypothesis that peripheral blood CD38 bright $CD8^+$ TEM cells increase in patients with aGVHD before clinical symptoms and can predict aGVHD, we serially quantified CD38 bright $CD8^+$ TEM populations in pediatric and young adult allogeneic HSCT patients. We found that CD38 bright $CD8^+$ TEM cells were readily detected in patients before aGVHD and that peripheral blood elevation of these cells could be used to predict onset of aGVHD.

METHODS

Patients and Transplantation Procedures

Institutional review board approval was granted for this study and participants gave informed consent. Twice weekly blood samples were obtained from 49 consecutive pediatric and young adult patients who underwent allogeneic HSCT at our center between April, 2013 and April, 2014 and received a T cell–replete graft. The choice of conditioning regimen and GVHD prophylaxis was made by the treating physician. All patients received supportive care measures, including nutritional support, intravenous immunoglobulin replacement, antifungal, antiviral, and anti–*Pneumocystis jerovecii* prophylaxis, as part of routine clinical care. Clinical information was prospectively collected until day +100, including date of onset of aGVHD, stage and grade of aGVHD, and viral reactivation with Epstein-Barr virus, adenovirus, and cytomegalovirus. aGVHD was diagnosed clinically by the treating physician and supported with biopsies whenever indicated. aGVHD was graded using the modified Glucksberg criteria [39].

Quantification of Peripheral Blood CD38 Bright $CD8^+$ TEM

Venous blood samples were prospectively collected once before transplantation and twice weekly for 7 weeks after stem cell infusion for characterization of T cell phenotype. Freshly obtained blood samples were incubated with fluorochrome-conjugated monoclonal antibodies directed against CD3 (Biolegend, San Diego, CA), CD38 (eBioscience, San Diego, CA) CD45, CD8, CD45RA, and CCR7 (BD Biosciences, San Jose, CA), followed by red cell lysis and fixation. Samples were analyzed by flow cytometry on a FACS Canto II flow cytometer (BD Biosciences). Data were analyzed using FCS Express version 4.0 (De Novo Software). $CD8^+$ TEM cell populations were defined as $CD3^+$ $CD8^+$ lymphocytes that lacked expression of CD45RA and CCR7. Gating strategy is shown in Figure 1. The absolute whole blood $CD8^+$ T cell count was calculated from the percentage of $CD3^+$ $CD8^+$ lymphocytes within the lymphocyte gate (as determined by CD45 intensity and side light scatter characteristics) multiplied by the absolute lymphocyte count. The absolute lymphocyte count was obtained from the complete blood count from the same day as flow cytometry sample collection, in the clinical hematology lab at Cincinnati Children's Hospital Medical Center. Absolute CD38 bright $CD8^+$ TEM cell counts were calculated as percentages of the absolute $CD8^+$ T cell count.

Statistics

For sample size calculations, we used the Fisher exact test. For comparisons of patient demographic and transplantation characteristics, we used the 2-sided Student *t*-test to compare age and Fisher exact test to compare categorical variables. A receiver operating characteristic analysis was performed to estimate area under the curve and determine the optimum threshold value of CD38 bright $CD8^+$ TEM elevation to predict aGVHD. Evaluations were performed using XLSTAT software (Addinsoft, Paris, France). CD38 bright $CD8^+$ TEM cells exhibited a high incidence rate of zeros. To account for this, and to account for the intensity of the nonzero levels, a zero-inflated gamma regression was used for CD38 bright $CD8^+$ TEMs at each time point. Standard errors of the mean were found by applying the delta method to Fisher's information matrix. Corresponding 2-sided *P* values were generated from the t-distribution. All computations were done in SAS 9.3 (SAS Institute, Cary, NC). Gray's method was used to generate cumulative incidence curves to compare the cumulative incidence of aGVHD in patients with and without elevation of CD38 bright $CD8^+$ TEM above the threshold value using the cmprsk package in R (R Foundation for Statistical Computing, Vienna, Austria). As only 1 patient died during study follow-up and none experienced a disease relapse, no competing risk analysis was performed. Statistical significance was considered for $P < .05$.

RESULTS

Patients

Forty-nine consecutive pediatric and young adult allogeneic HSCT recipients were enrolled for study. Two patients were excluded from analysis as they did not have peripheral blood samples the week before diagnosis of aGVHD. Demographic and transplantation information for the remaining 47 patients are summarized in Table 2. The median age of patients who developed aGVHD was 13.5 years (range, 1 to 33 years) compared with 7.5 years (range, .25 to 25.5 years) in the non-aGVHD group ($P = .07$). Fifty-three percent of patients in the aGVHD group underwent transplantation for an underlying diagnosis of a malignancy, whereas 12% of patients underwent transplantation for a malignancy in the non-aGVHD group ($P = .02$). Sixty-one percent of patients in the aGVHD group received a myeloablative preparative regimen compared with 25% of patients

Table 2
Demographics of Patients with and without aGVHD

Factor	GVHD n = 23	No GVHD n = 24	*P* Value
Age, median (range), yr	13.5 (1-33)	7.5 (.25-25.5)	.07
Diagnosis			.02
Malignancy	12 (53%)	3 (12%)	
Primary immune deficiency	7 (31%)	11 (45%)	
Hemoglobinopathy	1 (4%)	1 (4%)	
Marrow failure	2 (8%)	8 (3%)	
Metabolic disorder	1 (4%)	1 (4%)	
Preparative regimen			.02
Myeloablative	14 (61%)	6 (25%)	
Reduced intensity	9 (39%)	18 (75%)	
Serotherapy in conditioning			.004
Alemtuzumab	3 (13%)	16 (66%)	
Antithymocyte globulin	15 (65%)	5 (20%)	
HLA match			.07
8/8	17 (74%)	22 (92%)	
7/8	6 (26%)	1 (4%)	
6/8	0 (0%)	1 (4%)	
Relation			.13
Related	6 (26%)	12 (50%)	
Unrelated	17 (74%)	12 (50%)	
Stem cell source			1.00
Bone marrow	22 (96%)	22 (92%)	
PBSC	1 (4%)	1 (4%)	
Cord blood	0 (0%)	1 (4%)	
GVHD prophylaxis			.22
CSA/MP	9 (39%)	16 (67%)	
CSA/MTX	8 (35%)	4 (17%)	
CSA/MMF	4 (18%)	2 (8%)	
Tacrolimus/MP/MVR	1 (4%)	0 (0%)	
MMF	0 (0%)	1 (4%)	
CSA	1 (4%)	1 (4%)	
Cell dose, median (range)			
TNC (10^8/kg)	6.4 (2.5-12.6)	6.6 (.7-19.4)	.21
CD34 (10^6/kg)	5.3 (1.1-16)	5.1 (.3-20.1)	4.60
CD3 (10^7/kg)	7.3 (2.3-28)	6.0 (2.3-20.2)	.51

PBSC indicates peripheral blood stem cells; CSA, cyclosporine A; MP, methylprednisolone; MTX, methotrexate; MMF, mycophenolate mofetil; MVR, maraviroc; TNC, total nucleated cell.

Table 3
Grade and Timing of aGVHD Relative to Absolute CD38 Bright CD8+TEM cells/μL before Onset of aGVHD

Patient	Maximum Absolute CD38 Bright CD8$^+$ TEM (cells/μL)	Percentage of CD38 Bright CD8$^+$ TEM Cells Corresponding to the Maximum Absolute Value (%)	Timing of Maximum Absolute CD38 Bright CD8$^+$ TEM (Day after HSCT)	Onset of aGVHD (Day after HSCT)	Onset of CD38 Bright CD8+ TEM >35 cells/μL (Day after HSCT)	Maximum Grade of aGVHD	Organ(s) Involved
1	64	77	26	33	26	1	Skin
2	69	92	48	58	37	3	GI, liver
3	32	88	34	41	34	1	Skin
4	304	87	34	47	20	1	Skin
5	231	98	7	34	7	1	Skin
6	41	84	24	34	24	2	Skin
7	99	99	25	32	25	4	Skin, liver
8	98	99	16	23	16	3	Skin, GI
9	90	96	52	69	17	3	Skin, liver
10	162	98	37	52	20	3	Skin, GI, liver
11	42	82	33	42	33	3	Skin, GI
12	36	92	11	15	11	4	Skin, GI
13	82	96	15	29	15	4	GI
14	37	98	43	44	12	2	Skin
15	151	99	45	79	15	3	Skin, GI
16	63	95	23	33	23	4	Skin, GI
17	47	97	25	31	25	1	Skin
18	87	82	17	18	17	3	GI
19	69	86	56	59	56	1	Skin
20	2	87	25	28	NA	1	Skin
21	12	90	36	37	NA	1	Skin
22	70	99	28	38	21	1	Skin
23	128	97	42	45	28	3	Skin, GI

in the non-aGVHD group ($P = .02$). Ninety-two percent of patients in the non-GVHD group received a graft from an 8/8 HLA–matched related or unrelated donor, whereas 74% of patients in the aGVHD group received a graft from a fully matched donor ($P = .07$). Over 90% of patients in both groups received bone marrow grafts ($P = 1.00$).

Development of aGVHD

Grade 1 (n = 9), grade 2 (n = 2), grade 3 (n = 8), and grade 4 (n = 4) aGVHD were diagnosed in 23 patients at a median of 37 days after HSCT (range, 15 to 79 days), as shown in Table 3. Organ(s) of involvement included isolated skin GVHD (n = 11); isolated gastrointestinal (GI) GVHD (n = 2); skin and visceral (GI or liver) GVHD (n = 8); GI and liver GVHD (n = 1); and skin, liver, and GI GVHD (n = 1).

Quantification of CD38 Bright CD8$^+$ TEM Cells before aGVHD

Increased values of peripheral blood CD38 bright CD8$^+$ TEM cells were observed in patients who subsequently developed aGVHD (Figure 2). The levels of elevation during weeks 4 to 7 were significantly higher for patients who developed aGVHD compared with patients who did not develop aGVHD ($P < .05$). The maximum average absolute CD38 bright CD8$^+$ TEM cell count was 86 ± 15.1 cells/μL in patients before developing aGVHD, compared with 27 ± 14.2 cells/μL in patients who did not develop aGVHD ($P < .0001$).

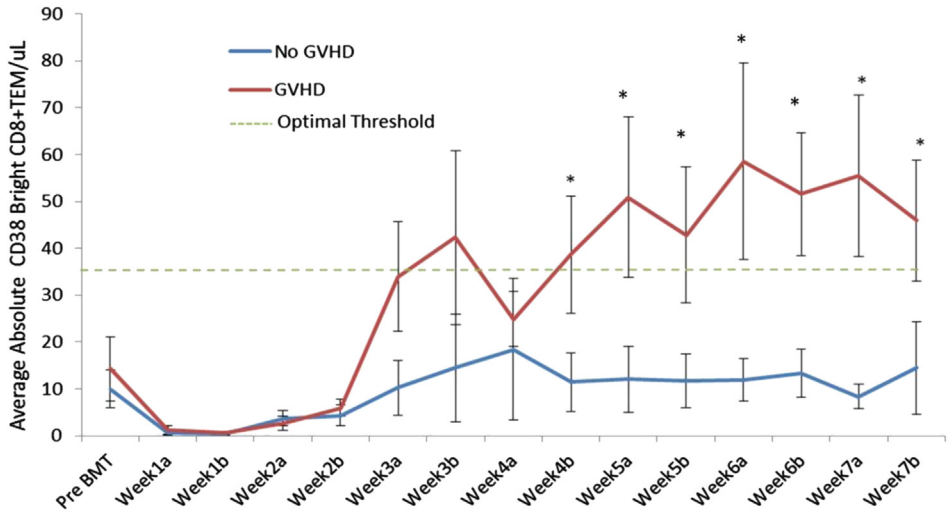

Figure 2. Kinetics of the average CD38 bright CD8$^+$ TEM cells/μL shown twice weekly in patients who developed acute graft versus host disease and in patients who did not develop acute graft-versus-host disease. $^*P < .05$. The error bars represent standard error of mean.

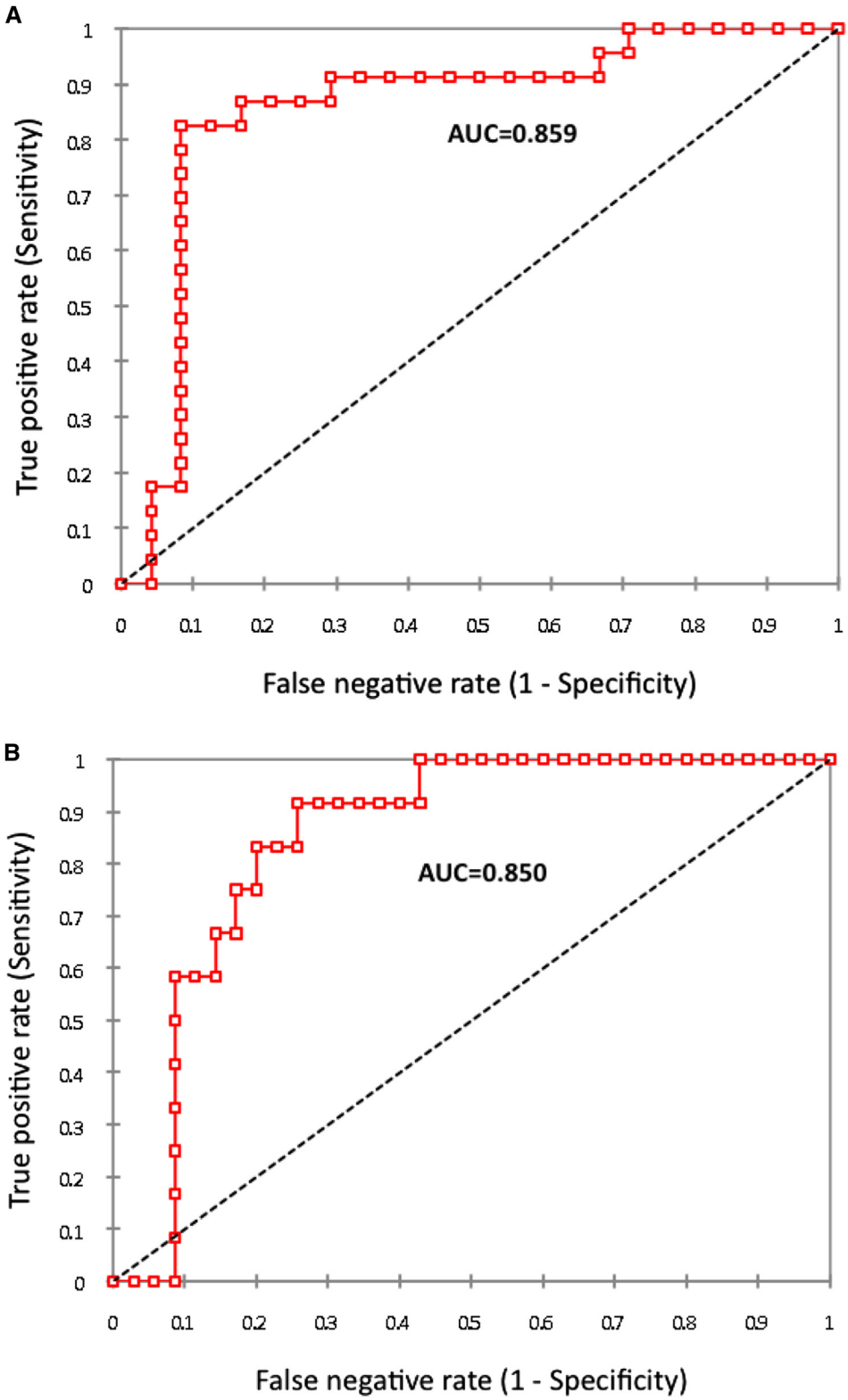

Figure 3. (A) Receiver operating characteristic curve analysis showing area under the characteristic curve of .859 for CD38 bright $CD8^+$ TEM cells/μL as a predictor of aGVHD. (B) Receiver operating characteristic curve analysis of grades 3 and 4 aGVHD versus grades 0 to 2 aGVHD showing an area under the curve of .850.

The maximum peripheral blood elevation of CD38 bright $CD8^+$ TEM cells was observed at a median of 8 days (range, 1 to 34 days) before onset of clinical aGVHD. We did not observe an association between the extent of TEM elevation and the grade of aGVHD (data not shown).

Diagnostic Accuracy

A receiver operating characteristic curve demonstrated an area under the curve of .859 (Figure 3A) and revealed that an optimum threshold value of absolute CD38 bright $CD8^+$ TEM greater than 35 cells/μL was predictive of aGVHD with a

sensitivity of 82.6% and a specificity of 91.6%. Twenty-one patients with aGVHD developed an absolute CD38 bright CD8$^+$ TEM >35 cells/μL at a median of 10 days (range, 1 to 64 days) before onset of aGVHD. The positive and negative predictive values were 90.5% and 84.6%, respectively. The diagnostic accuracy was calculated to be 87%. When patients with grades 3 and 4 aGVHD were compared with patients with grades 0 to 2 aGVHD, the receiver operating characteristic curve analysis revealed an area under the curve of .850 (Figure 3B). Using the previously mentioned optimal threshold value of >35 cells/μL, the sensitivity and specificity were 91.7% and 74.3%, respectively. The cumulative incidence of day +100 aGVHD in patients who demonstrated absolute CD38 bright CD8$^+$ TEM cells > 35 cells/μL was 90%, compared with 15% in patients who did not demonstrate values above 35/μL ($P < .0001$) (Figure 4). The cumulative incidence of day +100 aGVHD of grades 3 and 4 in patients who demonstrated absolute CD38 bright CD8$^+$ TEM cells > 35 cells/μL was 52%, compared with 4% in patients who did not demonstrate values above 35/μL ($P = .0002$).

Occurrence of Viral Reactivation

Viral infection is known to cause TEM cell expansion [40], so we compared the incidence of Epstein-Barr virus, cytomegalovirus, and adenovirus reactivation between patients with and without aGVHD to ensure that our results were not influenced by viral-mediated TEM cell expansion (Table 4). The occurrence of viremia(s) was documented until day +100 if no aGVHD were observed or until a diagnosis of aGVHD was made and immunosuppressive treatment was started. No statistically significant differences were observed between groups (Table 4). Notably, in patients who did not develop aGVHD but did develop viremia (n = 9), absolute CD38 bright TEM > 35 cells/μL occurred in only 1 patient. All remaining patients who developed viremia but not aGVHD (n = 8) maintained CD38 bright TEM populations of 35 cells/μL or less.

DISCUSSION

Here we present data on 47 pediatric allogeneic hematopoietic stem cell transplant recipients and show that increases of CD38 bright CD8$^+$ TEM cells to greater than 35 cells/μL predicted the onset of aGVHD approximately 1 week before clinical symptoms, with a sensitivity of 82.6%, a specificity of 91.7%, a positive predictive value of 90.5%, a negative predictive value of 84.6%, and a diagnostic accuracy of 87%. To our knowledge, this is the first report demonstrating the role of CD38 bright CD8$^+$ TEM cells as a predictive biomarker in aGVHD. The correlation between development of aGVHD and changes in other T cell subpopulations preceding onset of clinical symptoms has been described in the past but have generally lacked relevant levels of diagnostic screening accuracy [30]. A recent study in over 200 adult stem cell transplant recipients demonstrated elevated effector memory CD8$^+$ T cells on day +28 after transplantation before aGVHD [27]. An earlier study by Soiffer et al. described an increase in percentages and absolute CD8$^+$ T cell numbers that correlated with onset of aGVHD in CD6-depleted bone marrow recipients [28]. Gratama et al. described the correlation of early decreasing CD4 to CD8 ratios with increased risk of developing aGVHD in adult bone marrow transplant recipients [29]. These reports further corroborate the ability to use peripheral blood flow cytometric screening to identify patients at high risk of developing aGVHD. The quantification of CD38 bright CD8$^+$ TEM cells appears to elevate this approach to achieve clinically relevant levels of accuracy.

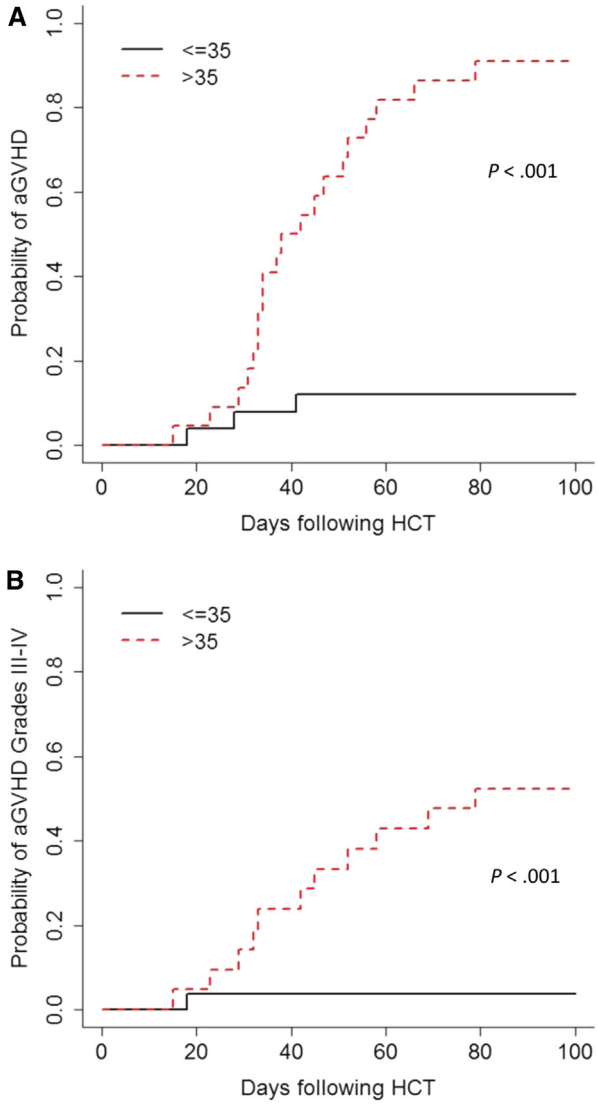

Figure 4. Probability of developing any grade of aGVHD (A) or grades 3 and 4 aGVHD (B) in patients who demonstrate absolute CD38 bright CD8$^+$ TEM cells/μL to >35 versus ≤35.

Our observations also might offer some novel insights into the biology of aGVHD. Naïve T cells travel to secondary lymphoid organs where they interact with antigen-presenting cells. Once activated, these T cells proliferate and generate effector cells that can migrate to inflamed tissues [21]. A number of chemokine receptors and adhesion molecules are involved in lymphocyte migration to secondary lymphoid organs or to peripheral tissues under homeostatic or inflammatory conditions. CCR7$^-$ memory T cells express high levels of tissue-specific receptors such as CD103, CLA, and beta 1 and 2 integrins, which are vital for migration to inflamed tissues [41,42]. Receptors of inflammatory chemokines such as CCR1, CCR3, and CCR5 are known to be selectively expressed in the effector memory subset [21]. As CD38 interacts with platelet endothelial cell adhesion molecule-1 and is the first step in leukocyte migration, the increased expression of CD38 on CD8$^+$ TEM cells might be related to trafficking of these cells to target organs [31].

Table 4
Occurrence of Viral Reactivation after HSCT

Viral Reactivation after HCT	aGVHD (n = 23)	No aGVHD (n = 24)	*P* Value
Epstein-Barr virus	5 (21%)	7 (29%)	.74
Adenovirus	0 (0%)	3 (12.5%)	.23
Cytomegalovirus	1 (4%)	5 (20%)	.18

The occurrence of viremia(s) was documented until day +100 if no aGVHD was observed, or until a diagnosis of aGVHD was made and immunosuppressive treatment was started.

Although this observation is preliminary, there could be a role of CD38 in the pathophysiology of aGVHD. As disruption of chemokines, such as CCR5, involved in T cell trafficking to target organs is an effective strategy in preventing aGVHD [43], it is conceivable that as anti-CD38 therapies become more available, these may present an attractive therapeutic option for future consideration.

Our method of screening for aGVHD demonstrates a shift in the paradigm of identifying aGVHD biomarkers. Our approach takes into account the very dynamic nature of aGVHD onset and diagnosis by prospectively sampling peripheral blood twice weekly after stem cell infusion, akin to viral surveillance in the post-transplantation period. This methodology was feasible in our patient population and the flow cytometry assay was rapid and, likely, easy to perform at most clinical facilities with flow cytometry expertise.

Our study does have some limitations. We recognize that our sample size is small and that our findings need to be validated in larger number of patients and to define a more definitive optimum threshold to use to identify patients at risk. Our small sample size did not allow us to adjust for covariates, such as age, underlying diagnosis, preparative regimen, HLA match, and age-related variations in the rate of immune recovery, which could alter our study conclusions. Although absolute CD38 bright $CD8^+$ TEM cell expansion appears to be predictive of onset of aGVHD, we were not able to predict severity of aGVHD in the current analysis. It is our hope that future larger studies will reveal strategies that can be used to stratify patients at higher risk of grades 3 and 4 GVHD and/or steroid-refractory aGVHD.

In conclusion, we have observed that peripheral blood CD38 bright $CD8^+$ TEM cells can predict aGVHD with high sensitivity and specificity in pediatric and young adult HSCT recipients. A subsequent large-scale trial is currently in progress to further validate this novel observation and better define a firm threshold value. Studies exploring functional characteristics of these cells are also being carefully conducted to shed further insight into the biology of aGVHD.

ACKNOWLEDGMENTS

The authors acknowledge Avni Patel and Kelly Lake for assistance with sample collection. They also acknowledge the patients, families, and clinical staff at Cincinnati Children's Hospital Medical Center.

Financial disclosure: The authors have nothing to declare.

Conflict of interest statement: There are no conflicts of interest to report.

REFERENCES

1. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373:1550-1561.
2. Chen YB, Cutler CS. Biomarkers for acute GVHD: can we predict the unpredictable? *Bone Marrow Transplant*. 2013;48:755-760.
3. Harris AC, Ferrara JL, Levine JE. Advances in predicting acute GVHD. *Br J Haematol*. 2013;160:288-302.
4. Miyamoto T, Akashi K, Hayashi S, et al. Serum concentration of the soluble interleukin-2 receptor for monitoring acute graft-versus-host disease. *Bone Marrow Transplant*. 1996;17:185-190.
5. August KJ, Chiang KY, Bostick RM, et al. Biomarkers of immune activation to screen for severe, acute GVHD. *Bone Marrow Transplant*. 2011;46:601-604.
6. Kitko CL, Paczesny S, Yanik G, et al. Plasma elevations of tumor necrosis factor-receptor-1 at day 7 postallogeneic transplant correlate with graft-versus-host disease severity and overall survival in pediatric patients. *Biol Blood Marrow Transplant*. 2008;14:759-765.
7. Liu D, Yan C, Xu L, et al. Diarrhea during the conditioning regimen is correlated with the occurrence of severe acute graft-versus-host disease through systemic release of inflammatory cytokines. *Biol Blood Marrow Transplant*. 2010;16:1567-1575.
8. Xiao B, Wang Y, Li W, et al. Plasma microRNA signature as a noninvasive biomarker for acute graft-versus-host disease. *Blood*. 2013;122:3365-3375.
9. Kaiser T, Kamal H, Rank A, et al. Proteomics applied to the clinical follow-up of patients after allogeneic hematopoietic stem cell transplantation. *Blood*. 2004;104:340-349.
10. Thiant S, Labalette M, Trauet J, et al. Plasma levels of IL-7 and IL-15 after reduced intensity conditioned allo-SCT and relationship to acute GVHD. *Bone Marrow Transplant*. 2011;46:1374-1381.
11. Thiant S, Yakoub-Agha I, Magro L, et al. Plasma levels of IL-7 and IL-15 in the first month after myeloablative BMT are predictive biomarkers of both acute GVHD and relapse. *Bone Marrow Transplant*. 2010;45:1546-1552.
12. Choi SW, Kitko CL, Braun T, et al. Change in plasma tumor necrosis factor receptor 1 levels in the first week after myeloablative allogeneic transplantation correlates with severity and incidence of GVHD and survival. *Blood*. 2008;112:1539-1542.
13. Willems E, Humblet-Baron S, Dengis O, et al. Elevations of tumor necrosis factor receptor 1 at day 7 and acute graft-versus-host disease after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *Bone Marrow Transplant*. 2010;45:1442-1448.
14. Paczesny S, Braun T, Lugt MV, et al. Three biomarker panel at day 7 and 14 can predict development of grade II-IV acute graft-versus-host disease. *[ASH Annual Meeting Abstracts] Blood*. 2010;116:675.
15. Vander Lugt MT, Braun TM, Hanash S, et al. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. *N Engl J Med*. 2013;369:529-539.
16. Zheng H, Matte-Martone C, Jain D, et al. Central memory CD8+ T cells induce graft-versus-host disease and mediate graft-versus-leukemia. *J Immunol*. 2009;182:5938-5948.
17. Grogan BM, Tabellini L, Storer B, et al. Activation and expansion of CD8(+) T effector cells in patients with chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2011;17:1121-1132.
18. Bolanos-Meade J, Wu J, Logan BR, et al. Lymphocyte phenotype during therapy for acute graft-versus-host disease: a brief report from BMT CTN 0302. *Biol Blood Marrow Transplant*. 2013;19:481-485.
19. Sun Y, Tawara I, Toubai T, Reddy P. Pathophysiology of acute graft-versus-host disease: recent advances. *Transl Res*. 2007;150:197-214.
20. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Ann Rev Immunol*. 2004;22:745-763.
21. Sallusto F, Lenig D, Forster R, et al. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999;401:708-712.
22. Zheng H, Matte-Martone C, Li H, et al. Effector memory CD4+ T cells mediate graft-versus-leukemia without inducing graft-versus-host disease. *Blood*. 2008;111:2476-2484.
23. Dutt S, Tseng D, Ermann J, et al. Naive and memory T cells induce different types of graft-versus-host disease. *J Immunol*. 2007;179:6547-6554.
24. Dutt S, Baker J, Kohrt HE, et al. CD8+CD44(hi) but not CD4+CD44(hi) memory T cells mediate potent graft antilymphoma activity without GVHD. *Blood*. 2011;117:3230-3239.
25. Chen BJ, Deoliveira D, Cui X, et al. Inability of memory T cells to induce graft-versus-host disease is a result of an abortive alloresponse. *Blood*. 2007;109:3115-3123.
26. Zhang Y, Joe G, Zhu J, et al. Dendritic cell-activated CD44hiCD8+ T cells are defective in mediating acute graft-versus-host disease but retain graft-versus-leukemia activity. *Blood*. 2004;103:3970-3978.
27. Podgorny PJ, Liu Y, Dharmani-Khan P, et al. Immune cell subset counts associated with graft-versus-host disease. *Biol Blood Marrow Transplant*. 2014;20:450-462.
28. Soiffer RJ, Gonin R, Murray C, et al. Prediction of graft-versus-host disease by phenotypic analysis of early immune reconstitution after CD6-depleted allogeneic bone marrow transplantation. *Blood*. 1993;82:2216-2223.
29. Gratama JW, Naipal A, Oljans P, et al. T lymphocyte repopulation and differentiation after bone marrow transplantation. Early shifts in the ratio between T4+ and T8+ T lymphocytes correlate with the occurrence of acute graft-versus-host disease. *Blood*. 1984;63:1416-1423.

30. Paz Morante M, Briones J, Canto E, et al. Activation-associated phenotype of CD3 T cells in acute graft-versus-host disease. *Clin Exp Immunol*. 2006;145:36-43.
31. Quarona V, Zaccarello G, Chillemi A, et al. CD38 and CD157: a long journey from activation markers to multifunctional molecules. *Cytometry B Clin Cytom*. 2013;84:207-217.
32. Bhan AK, Reinherz EL, Poppema S, et al. Location of T cell and major histocompatibility complex antigens in the human thymus. *J Exp Med*. 1980;152:771-782.
33. Miller JD, van der Most RG, Akondy RS, et al. Human effector and memory CD8+ T cell responses to smallpox and yellow fever vaccines. *Immunity*. 2008;28:710-722.
34. Malavasi F, Deaglio S, Damle R, et al. CD38 and chronic lymphocytic leukemia: a decade later. *Blood*. 2011;118:3470-3478.
35. Ortolan E, Arisio R, Morone S, et al. Functional role and prognostic significance of CD157 in ovarian carcinoma. *J Natl Cancer Inst*. 2010; 102:1160-1177.
36. Rosso R, Fenoglio D, Terranova MP, et al. Relevance of CD38 expression on CD8 T cells to evaluate antiretroviral therapy response in HIV-1-infected youths. *Scand J Immunol*. 2010;71:45-51.
37. Savarino A, Bottarel F, Malavasi F, Dianzani U. Role of CD38 in HIV-1 infection: an epiphenomenon of T cell activation or an active player in virus/host interactions? *AIDS*. 2000;14:1079-1089.
38. Deaglio S, Mallone R, Baj G, et al. CD38/CD31, a receptor/ligand system ruling adhesion and signaling in human leukocytes. *Chem Immunol*. 2000;75:99-120.
39. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295-304.
40. Lugthart G, van Ostaijen-Ten Dam MM, Jol-van der Zijde CM, et al. Early cytomegalovirus reactivation leaves a specific and dynamic imprint on the reconstituting T cell compartment long-term after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2014;20:655-661.
41. Baggiolini M. Chemokines and leukocyte traffic. *Nature*. 1998;392: 565-568.
42. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. *Science*. 1996;272:60-66.
43. Reshef R, Luger SM, Hexner EO, et al. Blockade of lymphocyte chemotaxis in visceral graft-versus-host disease. *N Engl J Med*. 2012; 367:135-145.